Speciation analysis of trace elements in foodstuffs by HPLC- and GC-ICPMS

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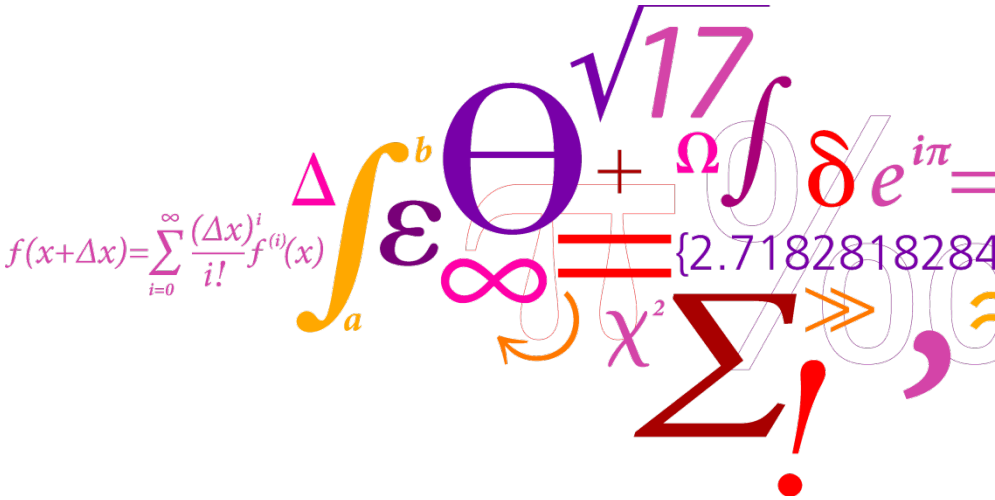
Jens J. Sloth

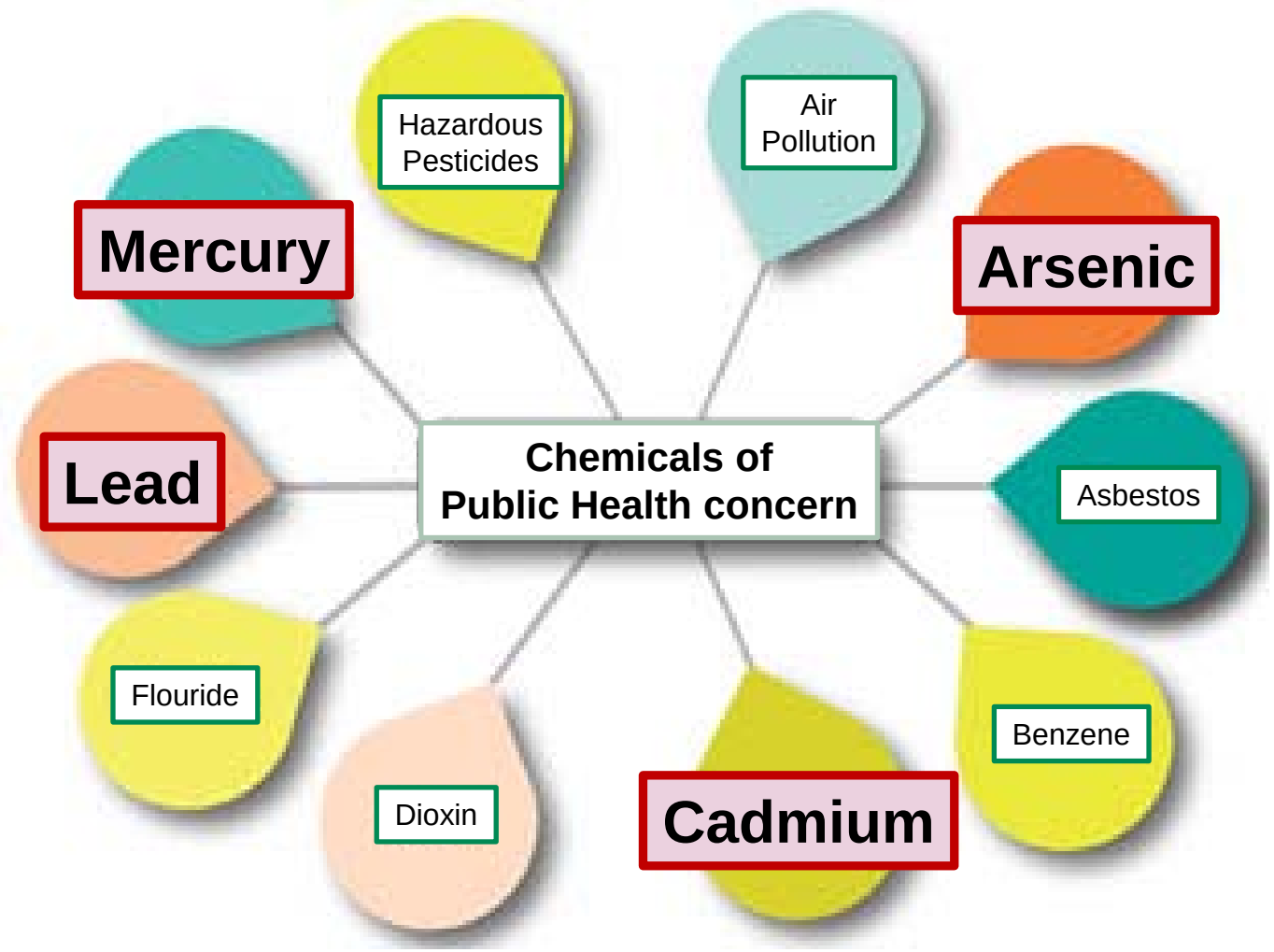


DTU Food
National Food Institute

The International Programme on Chemical Safety (IPCS)

WHO ILO UNEP

DTU



4 metals on the WHO IPCS list of top-10 priority chemicals of public concern

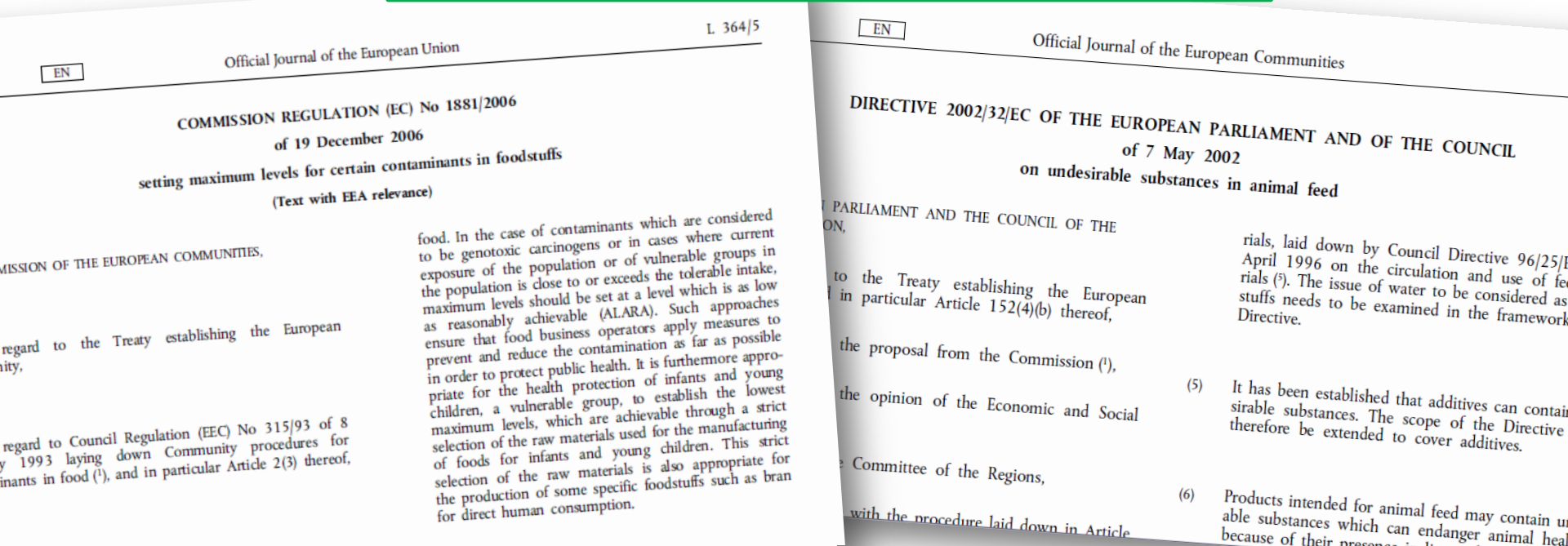
http://www.who.int/ipcs/

CURRENT SITUATION IN EU LEGISLATION:

Foodstuffs
MLs for Pb, Cd, Hg and Sn
EU directive 2006/1881/EC

Animal feedingstuffs
MLs for As, Pb, Cd and Hg
EU directive 2002/32/EC

Only maximum levels for
TOTAL CONCENTRATION
of the metals



EN Official Journal of the European Union L 364/5

COMMISSION REGULATION (EC) No 1881/2006
of 19 December 2006
setting maximum levels for certain contaminants in foodstuffs
(Text with EEA relevance)

MISSION OF THE EUROPEAN COMMUNITIES,

regard to the Treaty establishing the European
nity,

regard to Council Regulation (EEC) No 315/93 of 8
y 1993 laying down Community procedures for
inants in food [1], and in particular Article 2(3) thereof,

food. In the case of contaminants which are considered to be genotoxic carcinogens or in cases where current exposure of the population or of vulnerable groups in the population is close to or exceeds the tolerable intake, maximum levels should be set at a level which is as low as reasonably achievable (ALARA). Such approaches ensure that food business operators apply measures to prevent and reduce the contamination as far as possible in order to protect public health. It is furthermore appropriate for the health protection of infants and young children, a vulnerable group, to establish the lowest maximum levels, which are achievable through a strict selection of the raw materials used for the manufacturing of foods for infants and young children. This strict selection of the raw materials is also appropriate for the production of some specific foodstuffs such as bran for direct human consumption.

EN Official Journal of the European Communities

DIRECTIVE 2002/32/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL
of 7 May 2002
on undesirable substances in animal feed

PARLIAMENT AND THE COUNCIL OF THE
ON,

to the Treaty establishing the European
in particular Article 152(4)(b) thereof,

the proposal from the Commission [1],

the opinion of the Economic and Social

Committee of the Regions,

with the procedure laid down in Article

rials, laid down by Council Directive 96/25/E
April 1996 on the circulation and use of fe
rials [5]. The issue of water to be considered as
stuffs needs to be examined in the framework
Directive.

(5) It has been established that additives can contain
sirable substances. The scope of the Directive
therefore be extended to cover additives.

(6) Products intended for animal feed may contain u
able substances which can endanger animal hea
because of their presence

Analysis of total content vs. speciation analysis

Analysis of ~~total concentration~~

- **Arsenic**
- **Tin**
- **Mercury**
- **Lead**
- **Chromium**
- **Selenium**
- **Iodine**

Aim: get information on e.g.:

- biological activity
- toxicity
- mo
- bio
- lifetime, fate and metabolism
- chemical and physical activity

Speciation analysis is required!!

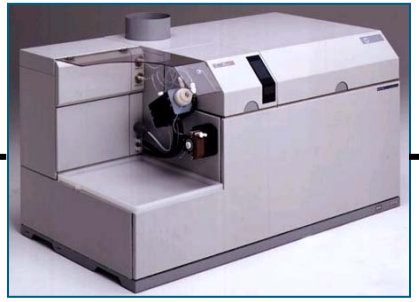
these characteristics are species related

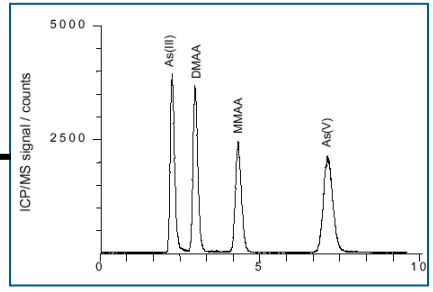
Speciation analysis – chromatography and element-specific detection

Sample extraction

Sample preparation

Sampling





HPLC / GC / CE

Sample introduction

Column

Separation

ICPMS

Element specific detection

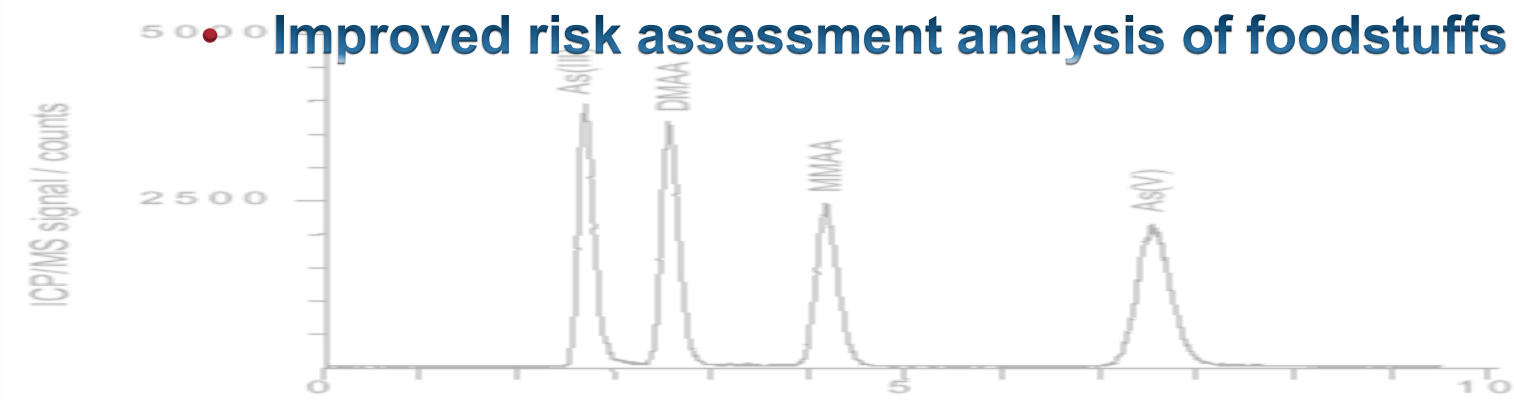
Result

Chromatogram

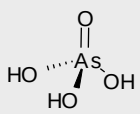
EXAMPLES

- **Arsenic** speciation analysis by HPLC-ICPMS
- **Organotin** speciation analysis by GC-ICPMS
- **Selenium** speciation by HPLC-ICPMS
- **Nanoparticle** determination by FFF-MALS-ICPMS

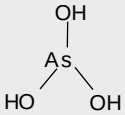
- Beyond total element determination
- Species-specific information
- Improved risk assessment analysis of foodstuffs



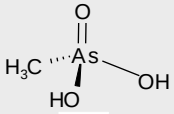
Arsenic compounds in the marine environment



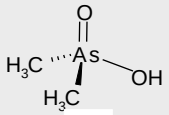
As(V)



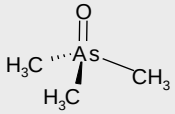
As(III)



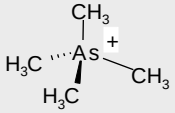
MA



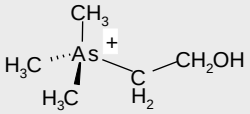
DMA



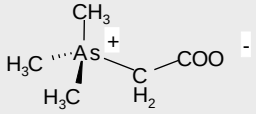
TMAO



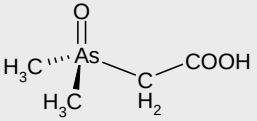
TETRA



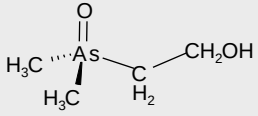
AC



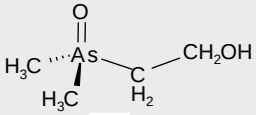
AB



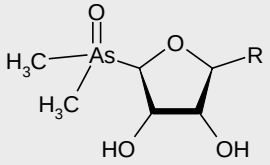
DMAA



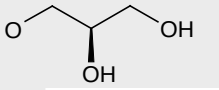
DMAE



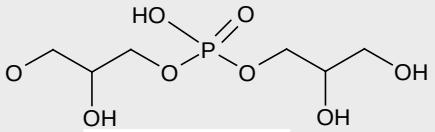
TMAP



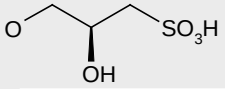
Dimethylarsinoylriboside



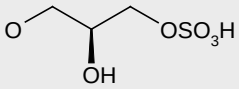
Dimethyl As-sugar 1



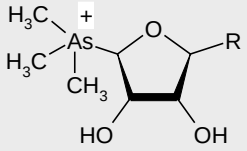
Dimethyl As-sugar 2



Dimethyl As-sugar 3



Dimethyl As-sugar 4

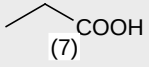


Trimethylarsonioriboside

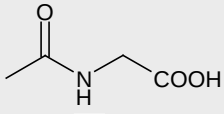
R = 1,2,3,4,5

(5)

(6)



(7)



(8)

More than **50** different arsenic species have been found in the marine environment

Speciation analysis of arsenic of scallop kidney

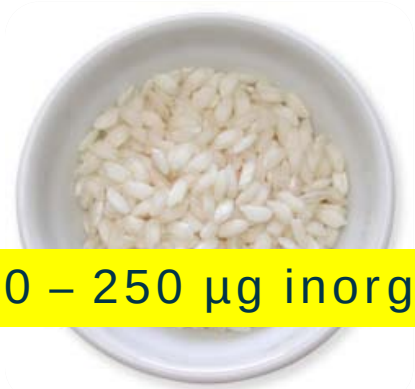
Cation-exchange with gradient elution – extraction with aqueous methanol

Column: Chrompack Ionospher 5C; Mobile phase: Pyridine; pH = 2.7

- seven compounds identified by coelution with available standards
- **16** non-identified peaks

Sloth et al, J.Anal.At.Spectrom., 2003, 18, 452-459

Example – arsenic speciation
Important for correct risk assessment



~ 40 – 250 µg inorg As

<10 µg inorg As

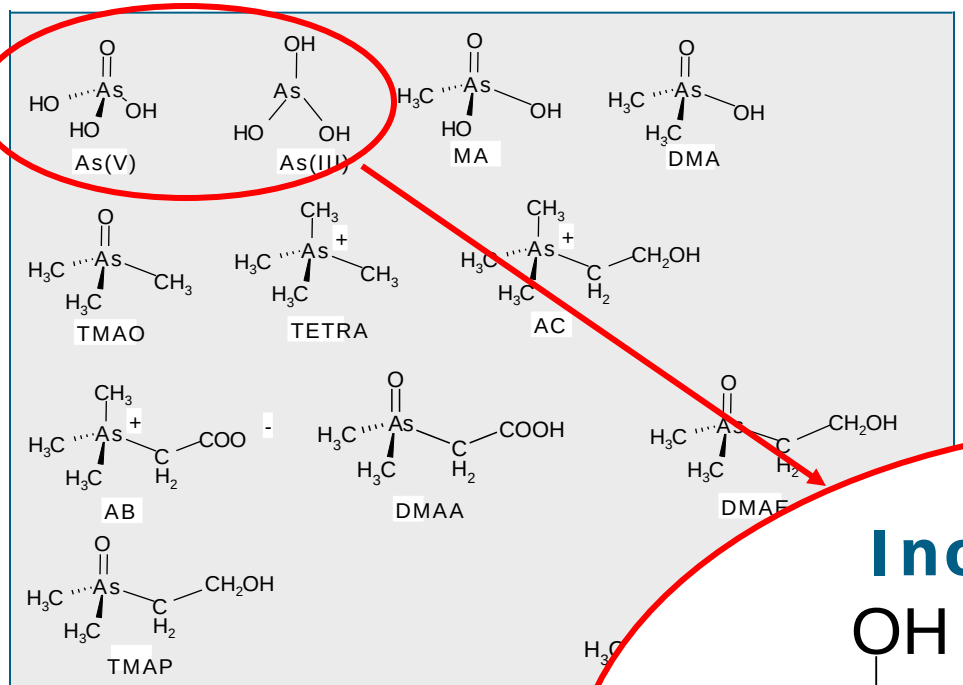
1 kg rice => 50-300 µg As

1 kg fish => 3000-10000 µg As

There is most focus on rice from a food safety point of view – why???

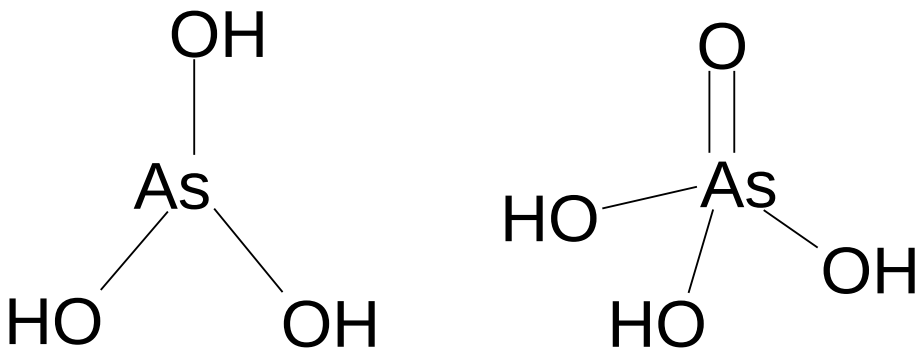
The chemical form of arsenic is important
=> Arsenic speciation

Arsenic compounds in the marine environment



Most toxic form of arsenic!!

Inorganic arsenic



EFSA (2009) and JECFA (2010) opinions on arsenic in food

- Old PTWI value (WHO, 1988) was withdrawn *(15 µg/kg bw/week)*

efsa European Food Safety Authority

- **NEW! $BMDL_{1.0}$ = 0.3 – 8 µg/kg bw per day for inorganic arsenic**
- *=> EU dietary exposures within this range for average and high level consumers*
- *=> Risk to some consumers cannot be excluded*

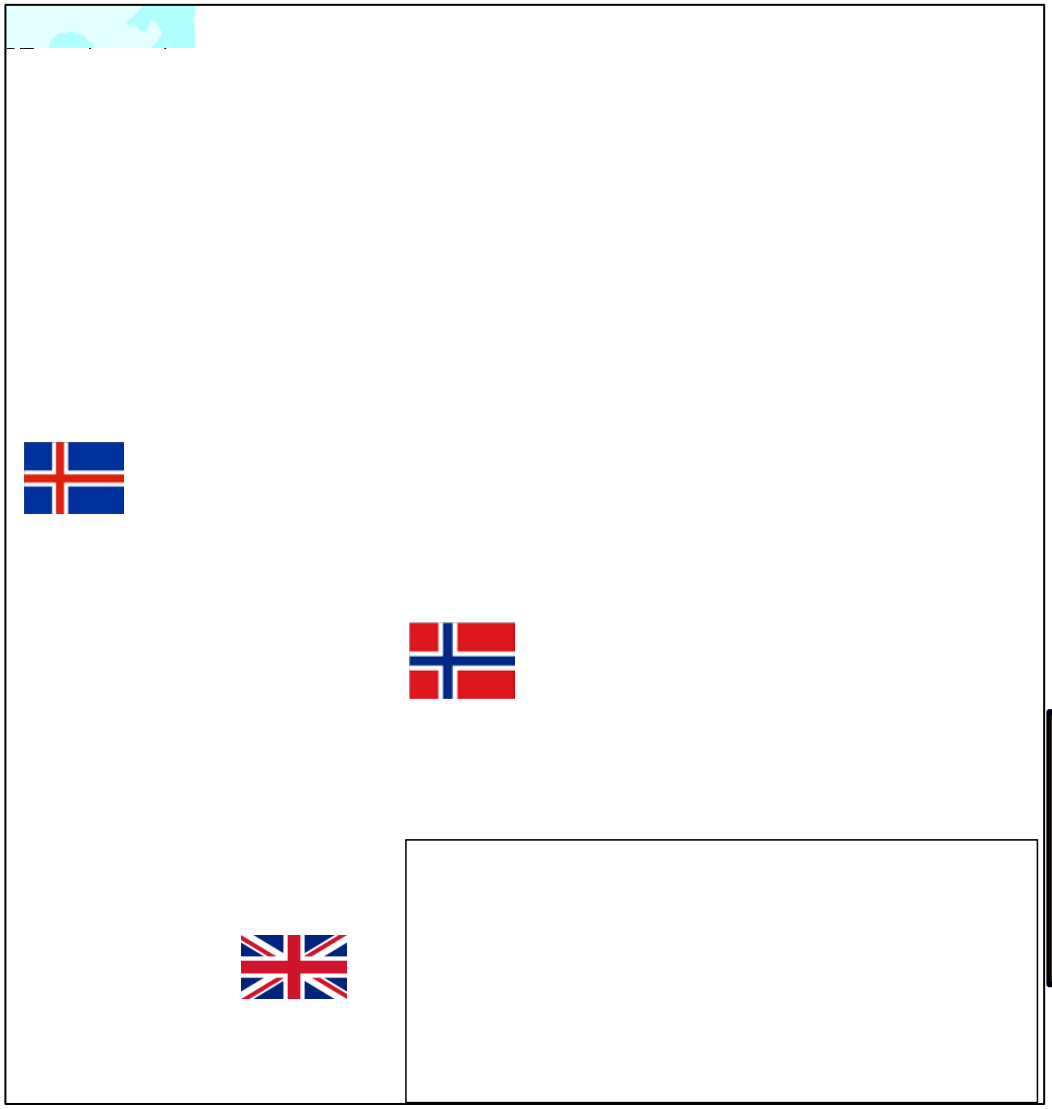
Food and Agriculture Organization of the United Nations

- **NEW! $BMDL_{0.5}$ = 3 µg/kg bw per day for inorganic arsenic**

  => 0.5% increased incidence of lung cancer for 12 y exposure

- *"…there is a need to produce speciation data for different food commodities to support dietary exposure assessment…"*
- *"…more accurate information on the inorganic arsenic content of foods is needed to improve assessments of dietary exposures to inorganic arsenic"*
- *"…need for validated methods for selective determination of inorganic arsenic in food matrices"*

Inorganic arsenic in wild caught fish => no concern



Norwegian survey

900 individual fish samples

- Atlantic halibut
- Cod
- Greenland halibut
- Mackerel
- Herring
- Tusk

Results

Total arsenic…….0.3-110 mg/kg

Inorganic arsenic…. < 0.01 mg/kg

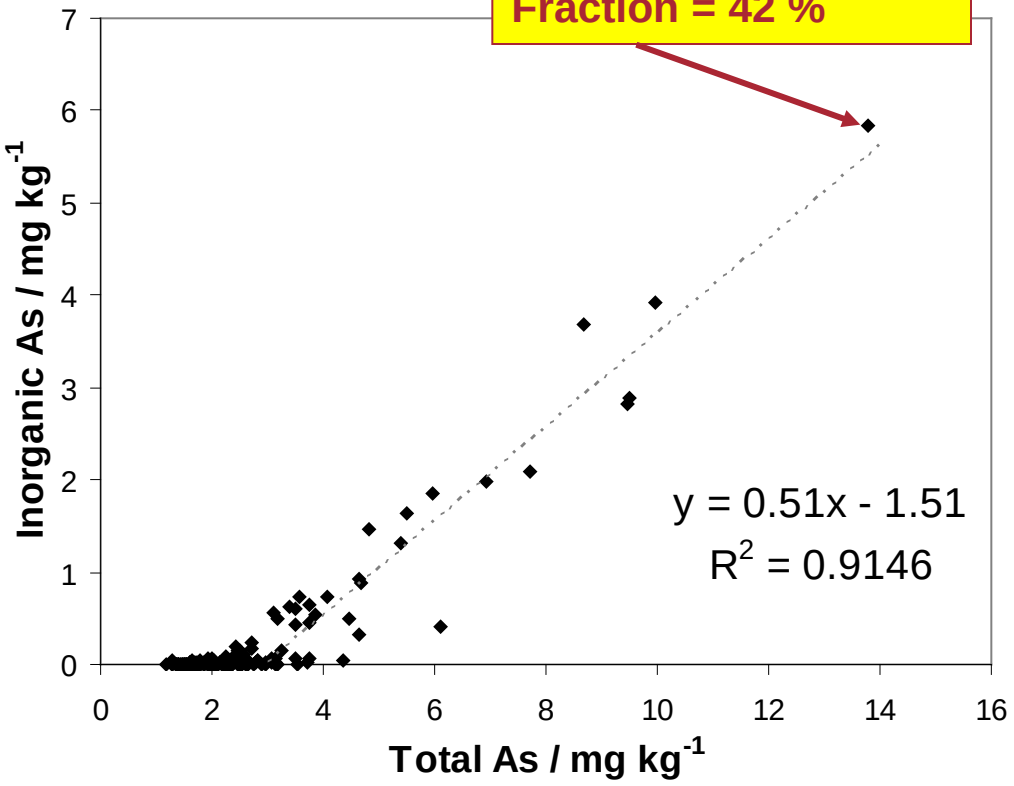
(only 37 samples > LOQ)

NIFES NATIONAL INSTITUTE OF NUTRITION AND SEAFOOD RESEARCH

Julshamn and Sloth, Fd Addit Contam B, 2012, submitted

...but in bivalves high contents in some samples...

Total As = 13.8 mg/kg
Inorg As = 5.8 mg/kg
Fraction = 42 %



Data from 175 blue mussel (*Mytilus edulis*) samples collected along the Norwegian Coastline.

NIFES
NATIONAL INSTITUTE OF NUTRITION AND SEAFOOD RESEARCH

Sloth and Julshamn, 2008, J. Agri.Food Chem., 56, 1269-1273

Arsenic in rice – an emerging health issue?

Environmental Pollution 152 (2008) 746–749

Rapid communication

Inorganic arsenic levels in baby rice are of concern

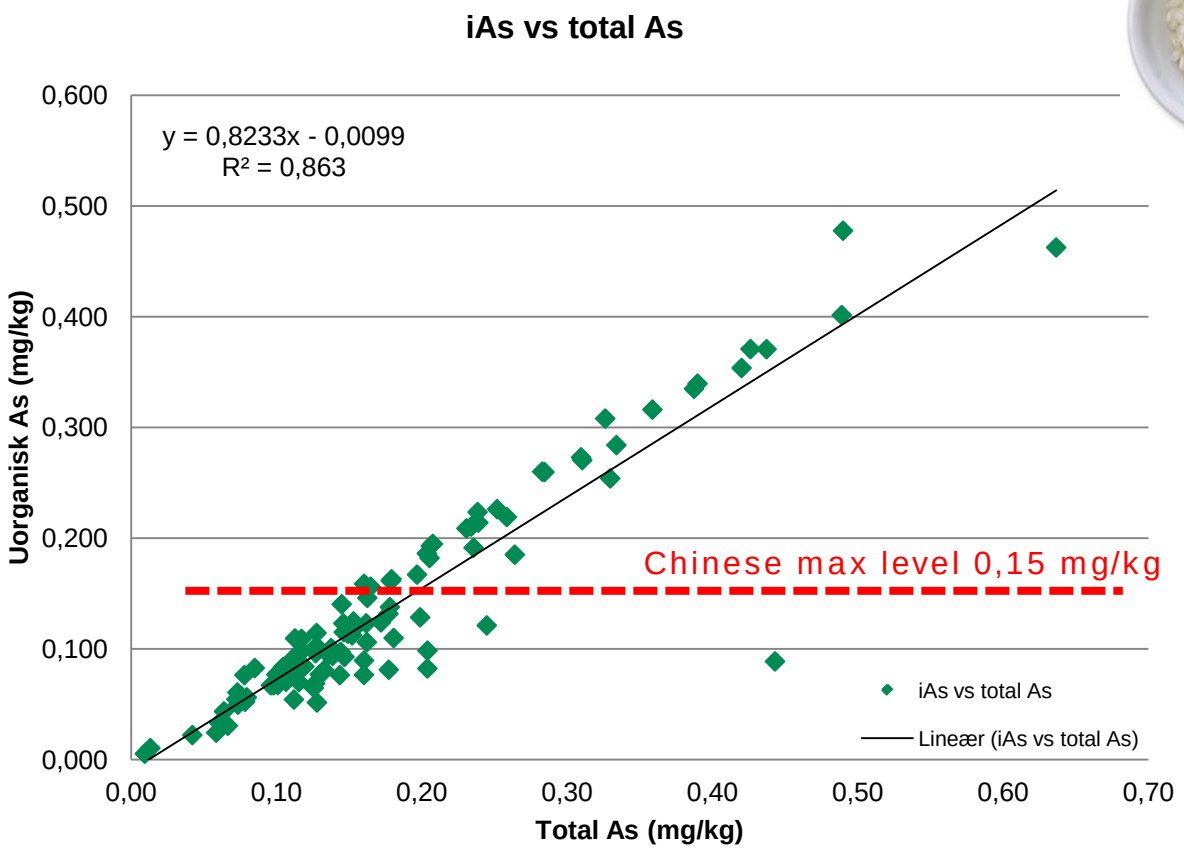
Andrew A. Meharg [a,*], Guoxin Sun [b], Paul N. Williams [a,b], Eureka Adomako [a], Claire Deacon [a], Yong-Guan Zhu [b], Joerg Feldmann [c], Andrea Raab [c]

[a] *School of Biological Sciences, University of Aberdeen, Cruickshank Building, St. Machar Drive, Aberdeen AB24 3UU, UK*
[b] *Research Centre for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China*

Median consumption of organic arsenic levels for UK babies from baby rice is above threshold considered safe.

- 17 samples from supermarkets in Aberdeen
- Total arsenic levels: 0.12 – 0.47 mg/kg
- Inorganic arsenic: 0.06 – 0.16 mg/kg (33 – 69 % of tAs)
- 35% above Chinese max level of 0.15 mg/kg iAs
- No regulation on As in food in EU (yet!)

Arsenic in rice products DK - survey



105 samples

-white rice
-brown rice
-red rice
-black rice
-rice crackers

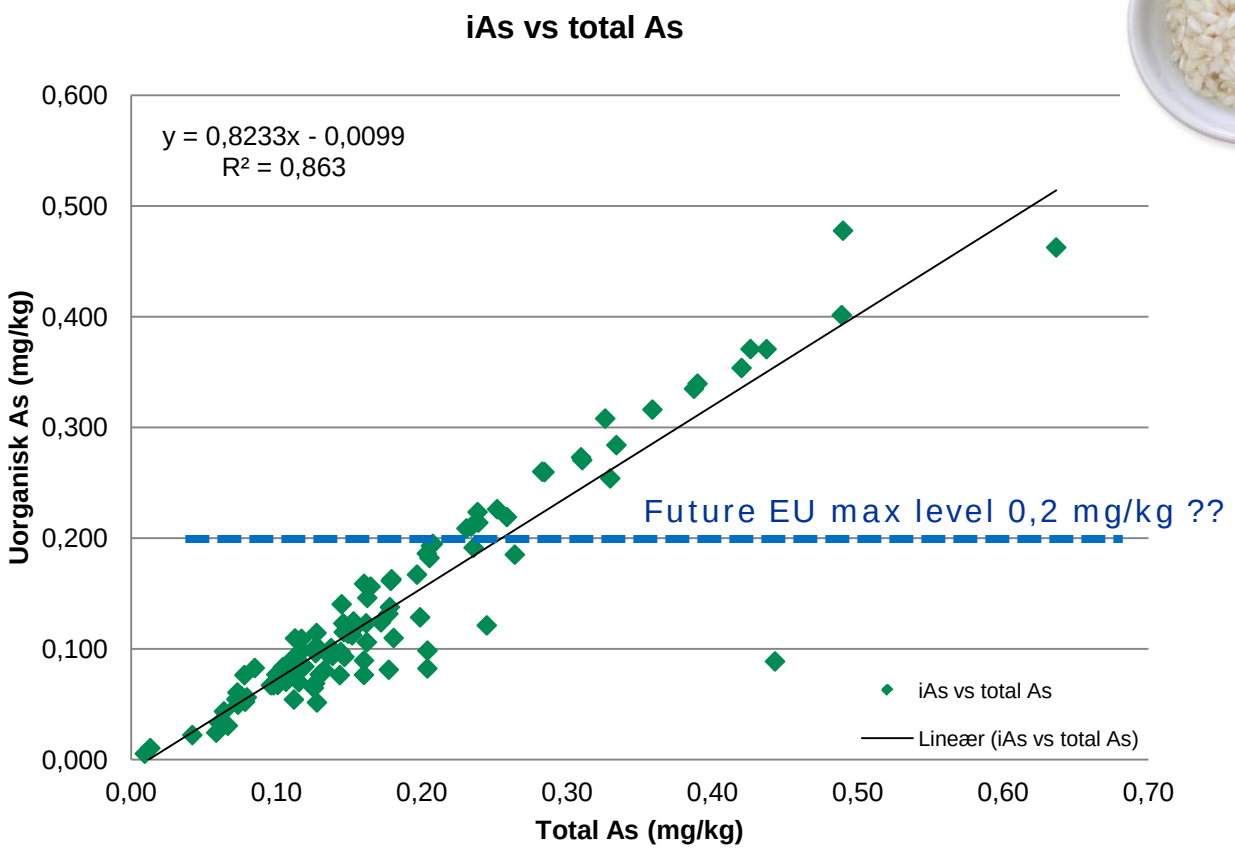
33 samples > 0,15 mg/kg

-2 parboiled (20%)
-4 brown (50%)
-4 red (50%)
-5 black (71%)
-1 Basmati (10%)
-1 Pudding rice (9%)
-1 wild rice (20%)
-15 rice crackers (100%)

Ministry of Food, Agriculture and Fisheries
Danish Veterinary and Food Administration

Sloth et al, in prep

Arsenic in rice products DK - survey

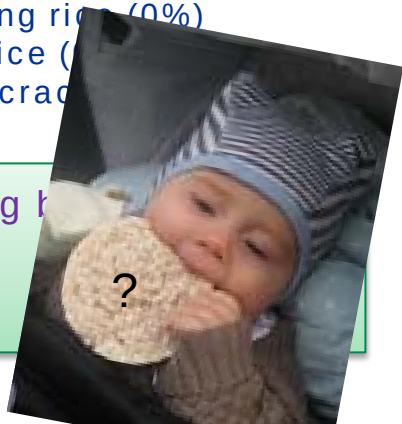


105 samples

-white rice
-brown rice
-red rice
-black rice
-rice crackers

22 samples > 0,2 mg/kg

-1 parboiled (10%)
-1 brown (12%)
-3 red (37%)
-2 black (28%)
-0 Basmati (0%)
-0 Pudding ri (0%)
-0 wild rice (
-15 rice crac



Rice cracker mean: 0.31 mg/kg – intake 50 g/dag => 15 µg iAs (~1 µg/kg b

> EFSA $BMDL_{01}$ 0.3-8 µg/kg bw/dag

Sloth et al, in prep

Inorganic arsenic in chinese food supplements

Name of Food supplement	Total Arsenic (μg/g)	Inorganic arsenic (μg/g)
Xiao Yao Wan	0.82	0.85
Bu Zhong Yi Qi Wan	0.62	0.50
Da Bu Yin Wan	0.59	0.55
Six Flavor teapills	0.72	N.D.
Golden Book Teapills	0.58	0.57
Xiang Sha Liu Jun Zi Wan	0.94	0.80
Gan Mao Ling	**1.24**	**1.01**
Chuan Xin Lian	5.00	3.17
Bi Yan Pian	0.70	0.58
Arouse power	1.12	1.02
Bio Chlorella	0.62	0.21
Unik Spirulina Kapsler	2.59	0.13
Chlorella	0.58	0.03
Ez-Biloba	0.63	0.67
Qvinde Dong Quai	0.68	0.48

Gan Mao Ling
(against flu and common cold)

Rec dose: 18 pills per day
⇒ iAs ~ 13 µg/day
⇒ 0.22 µg/kg bw/day (@60 kg)
Close to EFSA $BMDL_{01}$!!

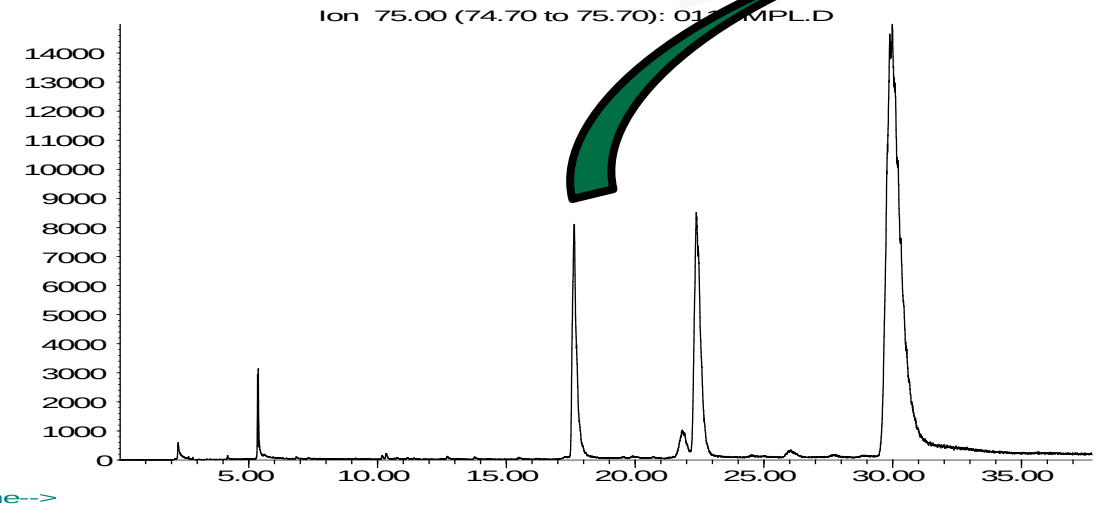
Hedegaard and Sloth, in prep

Arsenolipids – a class of lipidsoluble compounds

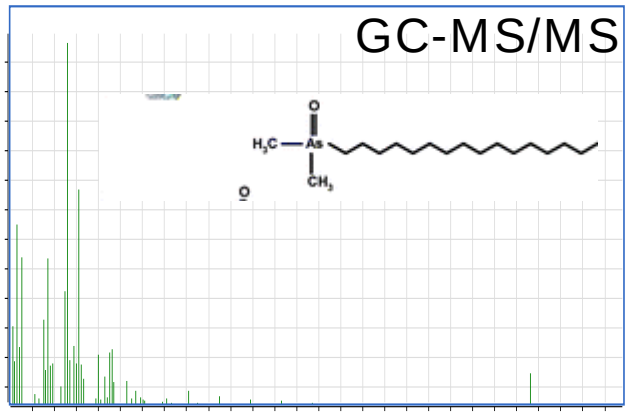
Sand eel oil *(important in fishfeed production)*

GC-ICPMS

Arsenic hydrocarbons



GC-MS/MS



- Still limited knowledge about:
  - chemical structures
  - biogenesis
  - bioavailibility, metabolism
  - toxicity??

Sele *et al*, Arsenolipids in marine oils and fats: A review, Food Chem, 2012

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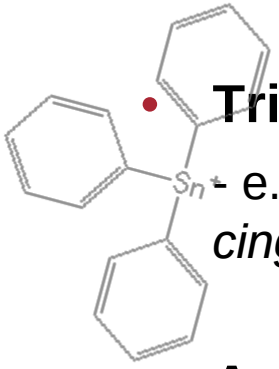
Uses of organotin compounds 1 - pesticides

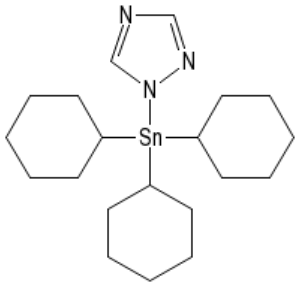


- **Triphenyltin acetate or hydroxide** (Brestan, Aquatin or Telostan)

  - e.g. used for control the population of brackish water snails *Cerithidea cingulata* in the pond culture of milkfish

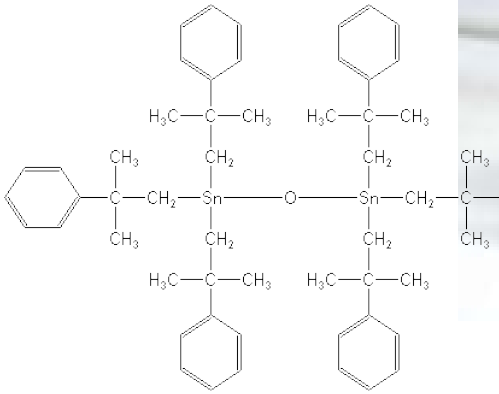
- **Acaricide azocyclotin**

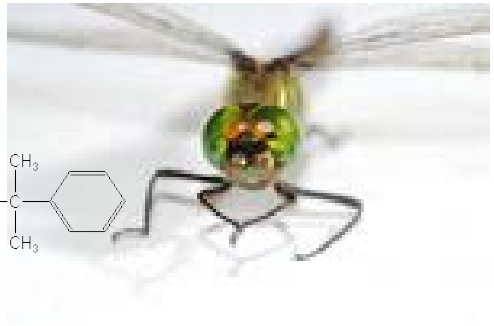
  - used against spiders, miters on e.g. be



- **Fenbutatin oxide**

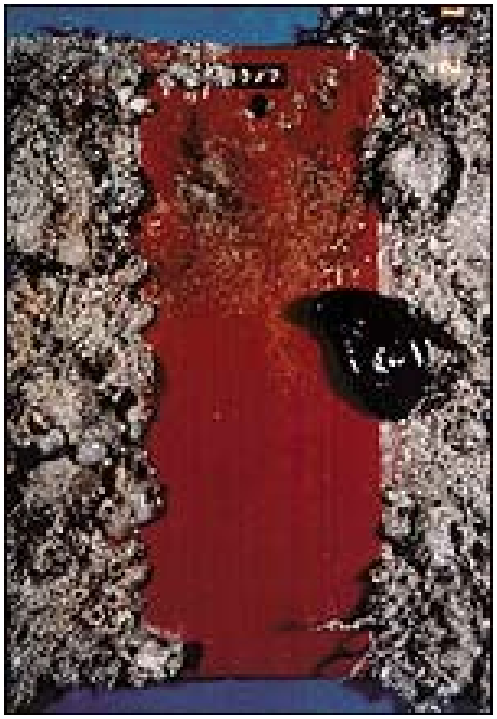
  - used as insecticide on almonds, grape





Uses of organotin compounds 2 - antifoulants

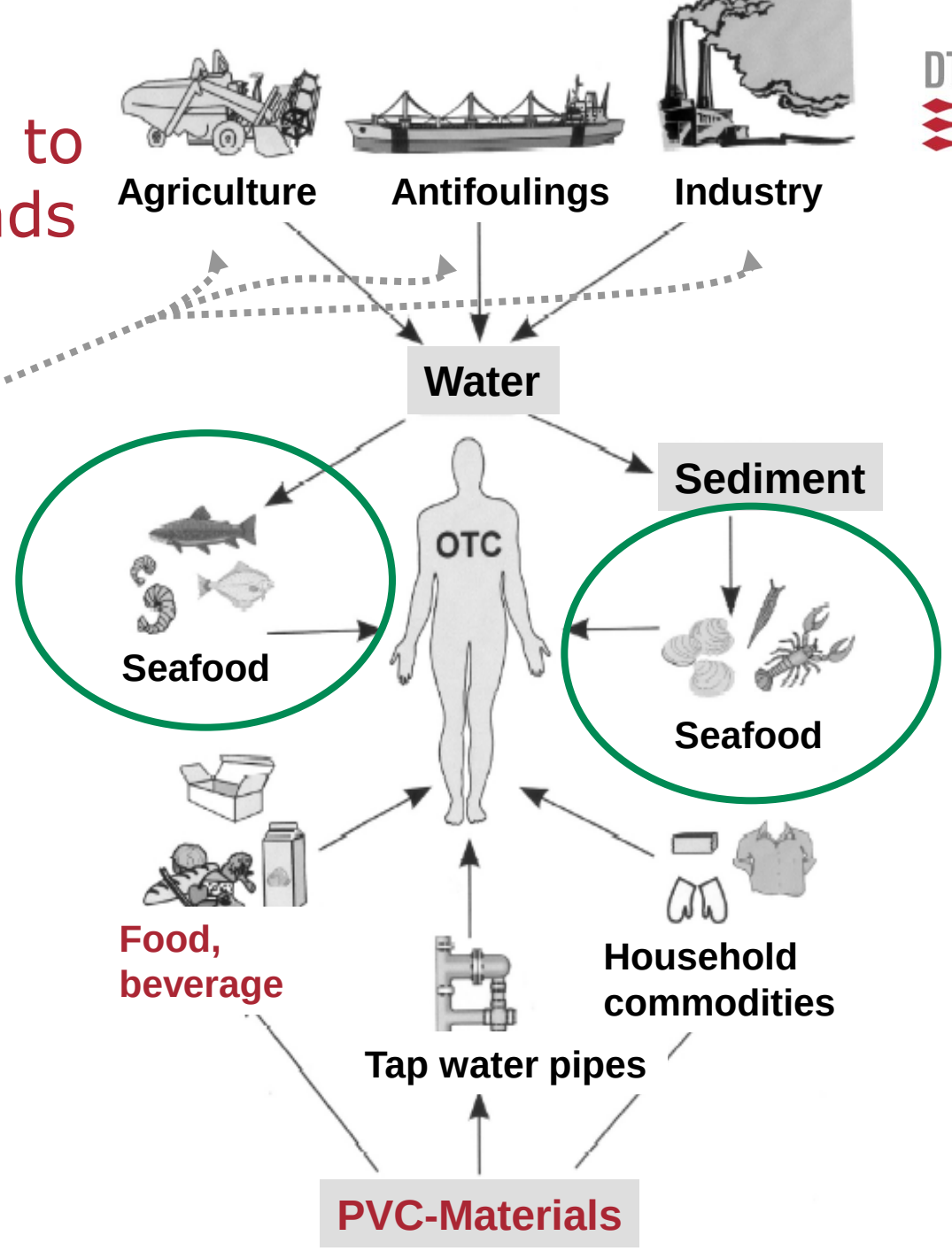
- Prevention of growth of plants and animals on ships and stationary marine constructions
- 1991: Banned on yachts <25 m in the Nordic countries
- 1999: Banned on yachts in all EU
- 2003: TBT ban on re-painting bigger ships with TBT paint
- 2008: TBT paint must be sealed or not used at all in EU ships



Example of metal plate treated with both antifouling paint and non-antifouling paint (www.ortepa.org)

Routes of exposure to organotin compounds

Used in
- **Agriculture**
- **Antifoulings**
- **Industry**



Hoch, Appl. Geochem., 2001, 719-43

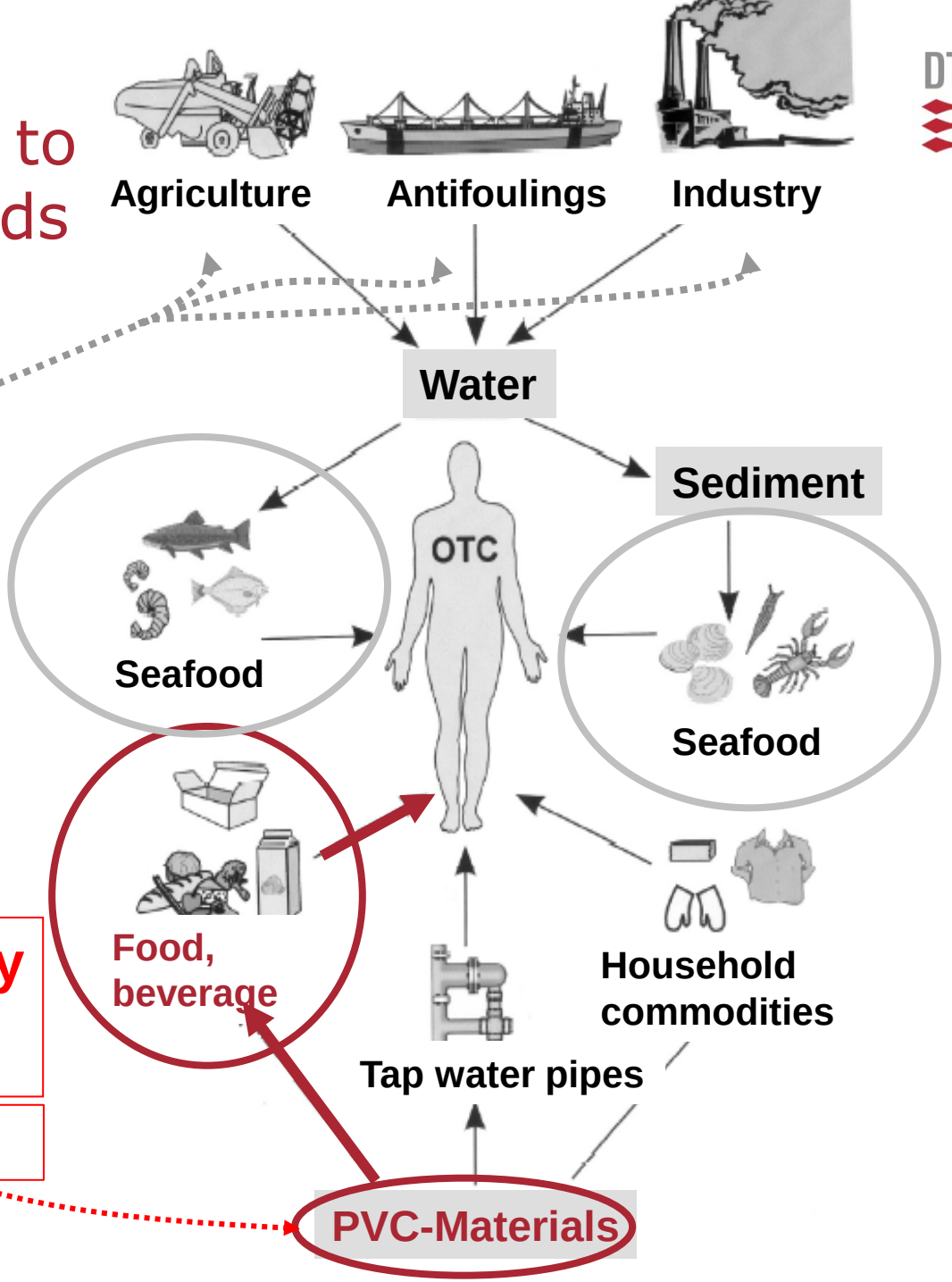
Data from seafood surveys at DTU Food - organotin compounds in marine samples

- **Project on various fishtypes:**
  - 214 samples of fish and shellfish from Danish fish wholesales
  - Generally sub-ppb levels found for all OT
  - Herring (up to 19 µg OT/kg)
  - Shark (up to 39 µg OT/kg)
  - Eel (up to 3200 µg OT/kg) !!

- **Projects on bivalves from Denmark:**
  - mono- , di- and tri-butylated and phenylated compounds
  - 100 bivalves samples/yr
  - generally only low levels found (sum OT < 10 µg/kg)

Routes of exposure to organotin compounds

Used in
- Agriculture
- Antifoulings
- Industry



- PVC-Materials

TDI: 0.25 µg/kg bw/day

∑ TBT, DBT, TPhT and DOT

=> ~17 µg/day @ bw 70kg

Hoch, Appl. Geochem., 2001, 719-43

Uses of organotin compounds 3 – PVC stabilisers

- PVC is a versatile polymer used worldwide for a variety of products
- At processing temperature PVC is unstable
- Stabilisers are added – functions against heat and light
- Mono- or di-alkyl tin compounds
- $RSnX_3$ and R_2SNX_2
- R = methyl-, butyl-, octyl-, dodecyl-
- X = mercaptoester, carboxylate, sulfide

pipes

containers

windows

cling films

Food Contact Materials

Applications of rigid and flexible PVC with OT

Rigid Applications	**Quantity (t/yr)**
Packaging, incl **food contact materials**, credit cards	12343
Rigid construction incl foamed sheeting	1016
Thin rigid film	290
Bottles	290
Pipes and moulding	290
Profile extrusions (e.g. windows)	290
Flexible Applications	
Flooring	312
Wallcovering	312
Steel coating	312
Miscellaneous (e.g. T-shirt printing)	156

Ref: ORTEP organisation

Legislation on OTCs in Food Contact Materials

Compounds	Maximum level (µg Sn/kg foodstuff)
∑ DBT, TBT, TPhT and DOT	40 (6)
∑ MMT, DMT	180
MOT	1200
MDDT	12000 (50)
DDDT	24000 (50)

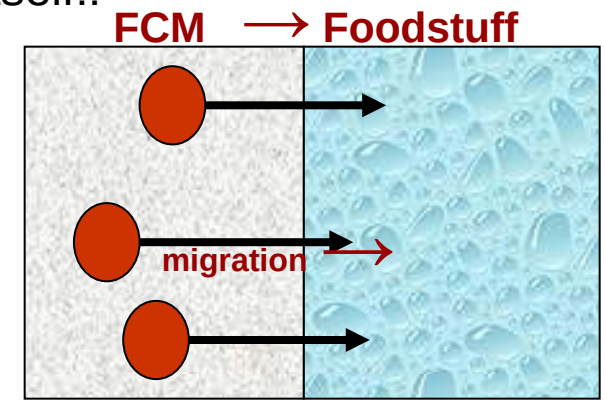
Ref: EFSA (2005); proposed EFSA values in parenthesis

Assumptions:

- 1 kg food per 6 dm^2

- 100 mL in contact with 0.6 dm^2

- Max levels on organotin migrating from the packaging material
- Testing by the use of food simulators (water, acid, oil, alcohol etc)
- BUT no maximum levels on organotins in the foodstuff itself!!

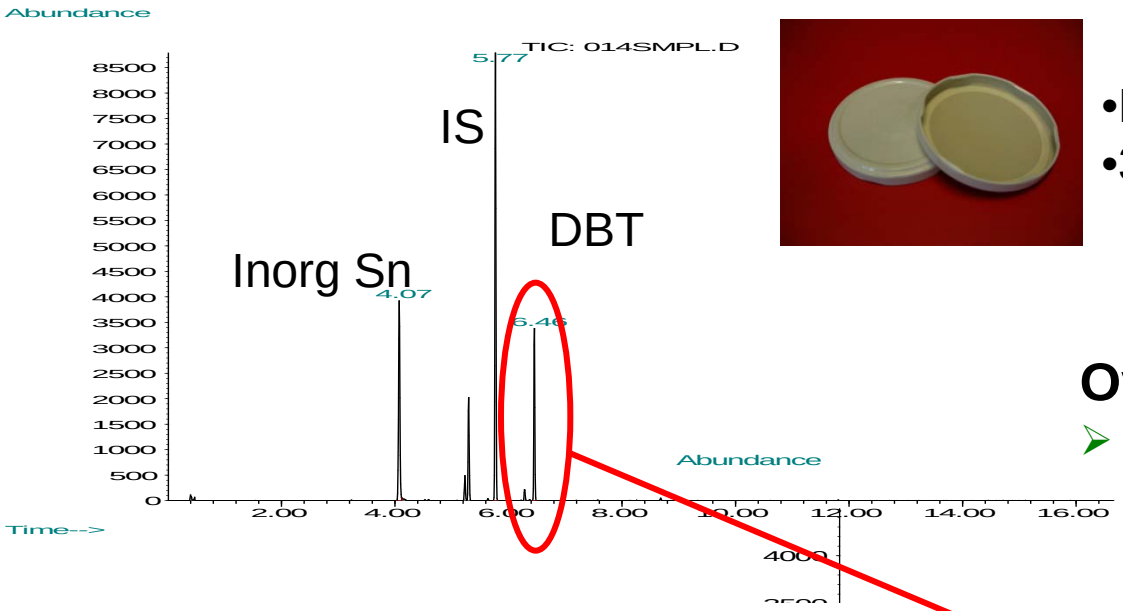


Organotin migration from Food Contact Materials II

Small scale survey on 33 FCMs

Baking paper, PVC cling films, silicone baking forms, lids with PVC gaskets

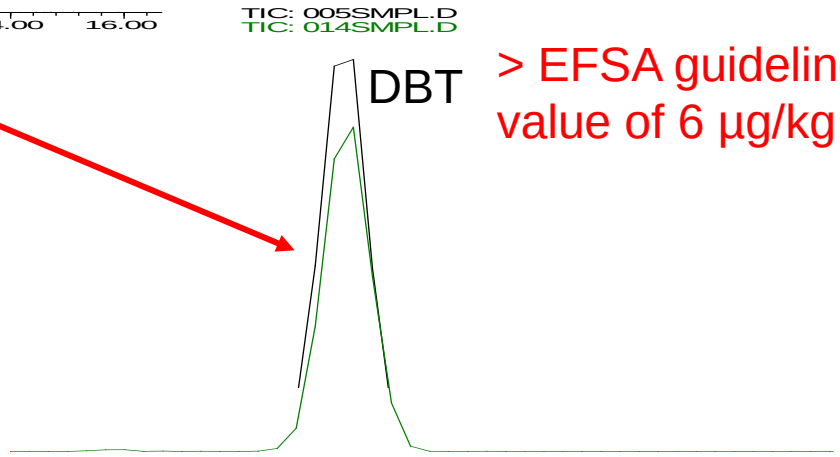
PUR-agglomerated cork wine stoppers



- PVC lid
- 3% acetic acid

Overlaid standard and sample

➢ DBT concentration: 9.9 µg/kg



> EFSA guideline value of 6 µg/kg

Output of DK survey:
-33 samples
-11 contained OT (mainly DBT)
-3 exceeded EFSA guideline limit

Sloth et al, in prep

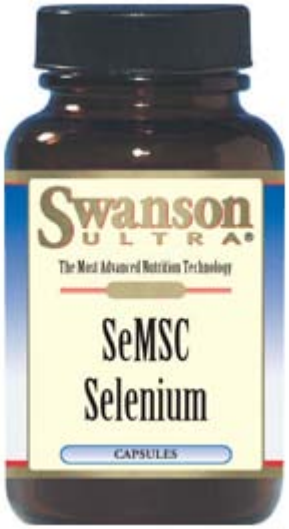
Selenium in commercial food supplements

Organic bound Se?

Se yeast?

Selenomethionine?

Selenite?



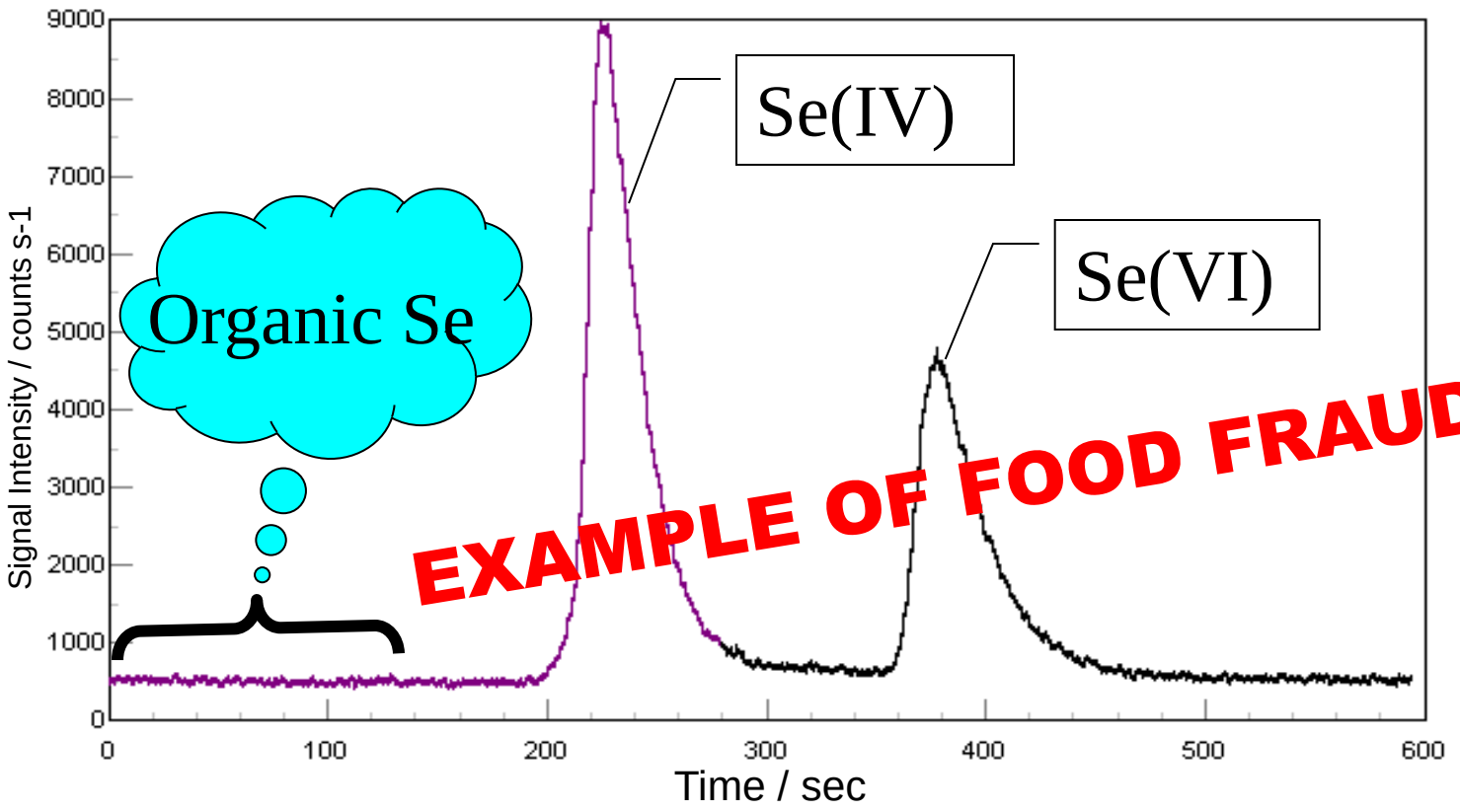
Amino chelated Se ?

Selenate?

Is the selenium source declared correctly??

Se speciation by HPLC-ICPMS

Supplement declared as *organic bound* Selenium (125 µg/tablet)



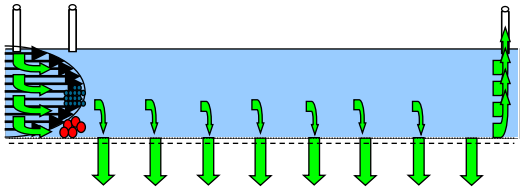
Larsen et al, unpublished data

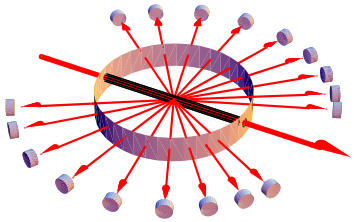
Silver nanoparticles in products related to food and beverages

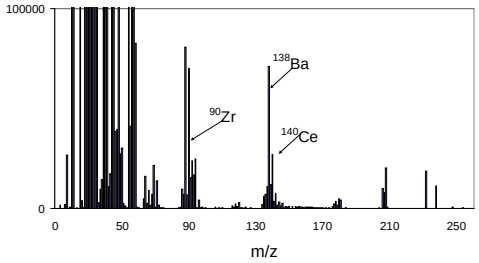
NanoSil SOLUTION

http://www.nanotechproject.org/

The analytical platform







Asymmetric flow field flow fractionation

Optical detection (multi angle and dynamic light scattering, UV and fluorescence)

Inductively coupled plasma mass spectrometry (ICP-MS)

Particle separation according to their size (small NPs elute first)

Particle detection (fractogram)

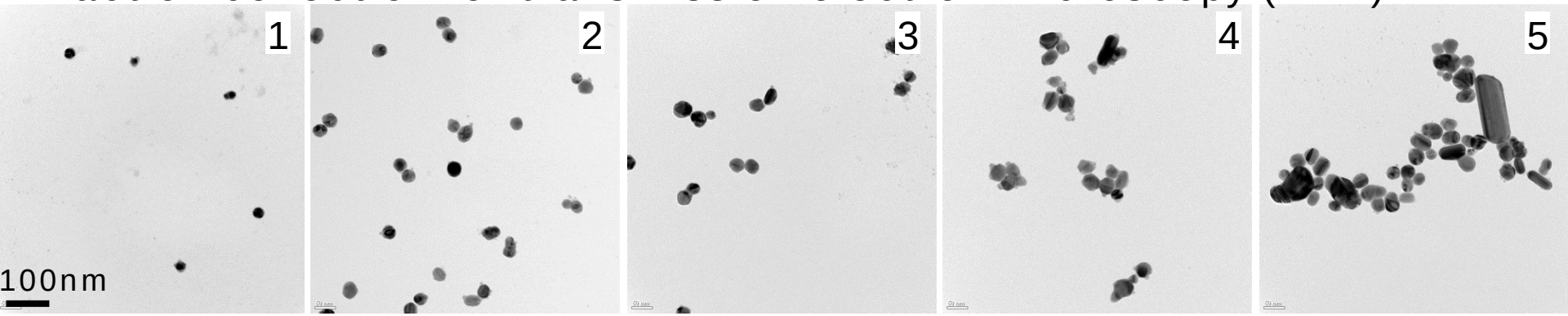
Size determination (root mean square, hydrodynamic and geometric radius)

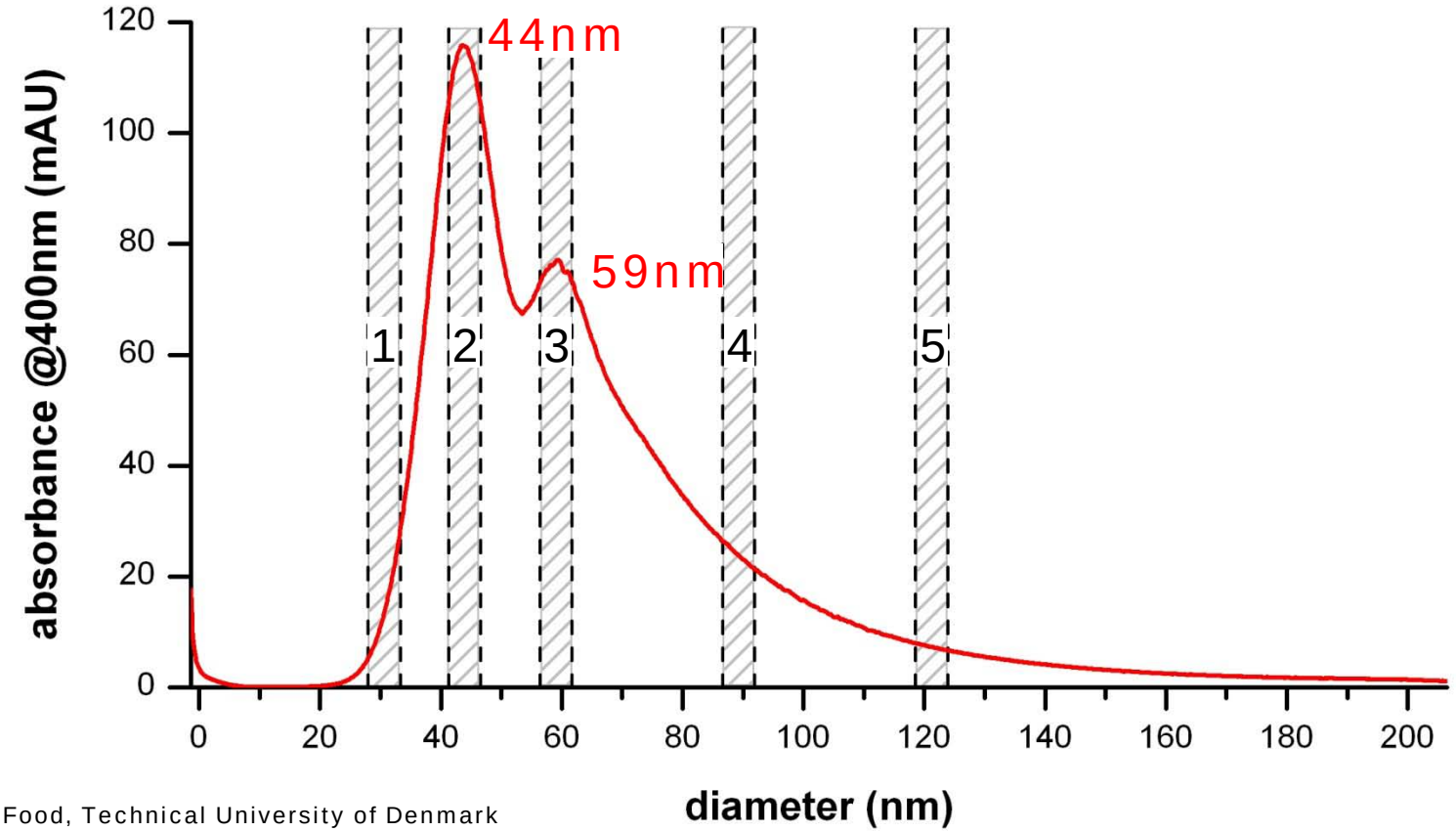
Elemental detection for identification of particles

Quantification

Determination of nanoparticle size distribution

Fraction collection for transmission electron microscopy (TEM)





Perspective – trace elements in nanoform

NanoLyse Project "Nanoparticles in Food: Analytical methods for detection and characterisation"

Validated methods for the determination of inorganic ENP in food extracts, based on size separation, size determination and specific detection

silver nanoparticles in lean meat

silica nanoparticles in tomato soup

http://www.nanolyse.eu

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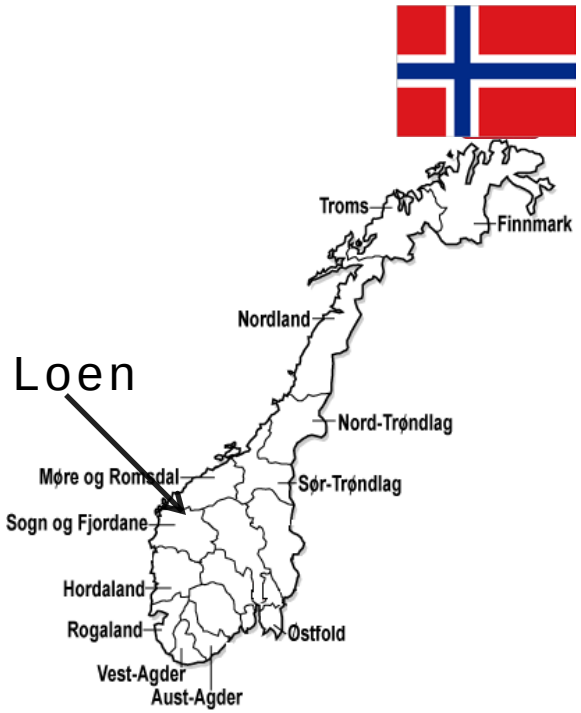
Thanks for your attention!



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